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Oxidative pyrimidine metabolism in *Rhodococcus erythropolis* useful for valuable nucleoside synthesis: Discovery of a novel amidohydrolase, ureidomalonase

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Abstract

Through the investigation of the oxidative pyrimidine metabolism in *Rhodococcus erythropolis*, a novel enzyme, ureidomalonase, catalyzing the final step of the metabolism was found. This enzyme catalyzed the amidohydrolysis of ureidomalononic acid, a product of barbiturase reaction, to urea and malonic acid. These finding brought a comprehensive understanding about oxidative pyrimidine metabolism for the first time, which is consisted of uracil/thymine dehydrogenase, barbiturase, and ureidomalonase.

Introduction

In a biological system, it is known that pyrimidines are metabolized through different pathways (Fig. 1). One is reductive pathway in which the initial reaction is reduction of pyrimidine to dihydropyrimidine. It is well recognized that mammals, plants, and microorganisms operate this pathway [1-3]. Whereas some microorganisms use the oxidative pathway in which the initial reaction is oxidation of pyrimidine, *e.g.*, uracil oxidation to barbituric acid [4-6]. Recently a novel pathway (rut pathway; pyrimidine utilizing) was reported, in which pyrimidine is converted to a final product, 3-hydroxypropionic acid [7,8]. Over the past several decades, the reports of oxidative pyrimidine metabolism are scarce. Although it is a naturally occurring metabolic pathway, the knowledge on the oxidative pathway and its biological importance is very limited. Before our study, only a few early works by three groups of scientists had been reported [4-6]. We initiated detailed studies on oxidative pyrimidine metabolism using uracil assimilating strain, *Rhodococcus erythropolis* JCM 3132. In this short communication, we describe the discovery of ureidomalonase, and discuss on the industrial potentials of the enzyme and whole pathway.

Results

Discovery of a novel amidohydrolase, ureidomalonase

In purification of barbiturase from *R. erythropolis* JCM3132, the barbituric acid decomposing activity was not associated with the urea producing activity [9]. An unknown product (which did not correspond to urea or malonic acid) was detected on HPLC analysis of the reaction mixture. Interestingly, urea was produced from barbituric acid when the following downstream fractions were added to the barbituric acid decomposing fractions. The downstream fraction itself catalyzed neither barbituric acid decomposition nor urea production directly from barbituric acid. These results suggested that the conversion of barbituric acid to urea involves two reaction steps.

As to the unknown product of barbiturase reaction, there are several observations: 1) it was spontaneously cyclized to barbituric acid; 2) it gave no absorption peak around 250 nm, therefore it was considered to be a non-cyclic compounds; 3) it was transformed to urea by the consecutive enzyme; and 4) malonic acid was co-produced with urea from it by the consecutive enzyme. These results indicates that the barbiturase reaction product is ureidomalonic acid, and that the consecutive enzyme is a novel amidase that catalyze amidohydrolysis of ureidomalonic acid to urea and malonic acid.

Purification, characterization, and gene cloning of ureidomalonase

We purified ureidomalonase to homogeneity from the soluble cell-free extracts of *R. erythropolis* JCM 3132, and the gene encoding the enzyme was cloned and expressed in *E. coli*. We confirmed that the *E. coli* transformant showed ureidomalonase activity, that is urea-forming activity from barbituric acid in combination with barbiturase. Sequence analysis of the gene revealed that ureidomalonase is grouped into GGCT (γ -glutamyl cyclotransferase) superfamily, and exhibited 99, 56, and 55% identity with putative amidases of *R. erythropolis* PR4 (Kyoto Encyclopedia Genes and Genomes accession No.;RER_58810), *Burkholderia ambifaria* AMMD (Bam_4083), *Pseudomonas aeruginosa* LESB58 (PLES_47201), respectively. Moreover, the gene cluster of oxidative pathway was found in comparison with the homologous (more than 99% homology) gene cluster in *R. erythropolis* PR4 genome sequence (RER_58760 to RER_58830), that was supposed to encode uracil/thymine dehydrogenase, uracil permease, aminotransferase, ureidomalonase, barbiturase, and uracil phosphoribosyl-transferase.

Discussion

Our work is the first detailed investigation on the oxidative pyrimidine metabolism. The

references on oxidative pyrimidine metabolism available are limited, and these reports showed that pyrimidine bases are initially oxidized to barbituric acid derivatives, and then the barbituric acid derivatives are further hydrolyzed to urea and malonic acid by barbiturase, directly [4-6]. However, these studies were performed with crude enzyme extracts, and the presented results were inadequate for confirming the direct conversion of barbituric acid to urea and malonic acid. A novel knowledge was given to physiology of this pathway by our present work. Through the understanding of oxidative pyrimidine metabolism at gene level, it becomes possible to control reaction equilibrium of pyrimidine degradation using genetic technique. The enzymes consisting oxidative pyrimidine metabolism are useful for equilibrium control of base exchange reaction for valuable nucleoside synthesis, for example, thymidine is converted to 2'-deoxyguanosine via 2-deoxyribose 1-phosphate catalyzed by pyrimidine nucleoside phosphorylase and purine nucleoside phosphorylase (Fig. 2). Addition of *R. erythropolis* JCM 3132 into above base exchange reaction, it is capable to remove free thymine out of the reaction system, and then the reaction equilibrium shift to direction for 2'-deoxyguanosine synthesis. Therefore, it is promising that improvement of the enzyme activities in oxidative pyrimidine metabolism by genetic engineering may contribute for increasing the productivity of useful nucleosides through base exchange reaction. Our discovery of ureidomalonase provides some insights into application of the oxidative pyrimidine metabolism and its physiological importance in nucleic acid metabolism.

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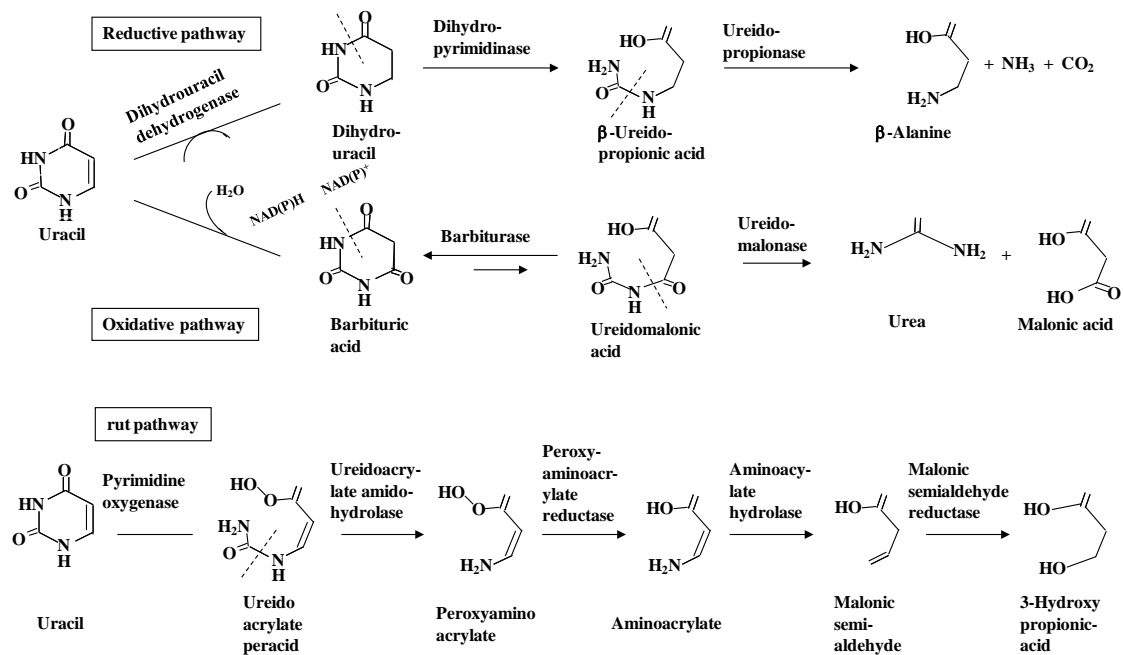


Fig. 1
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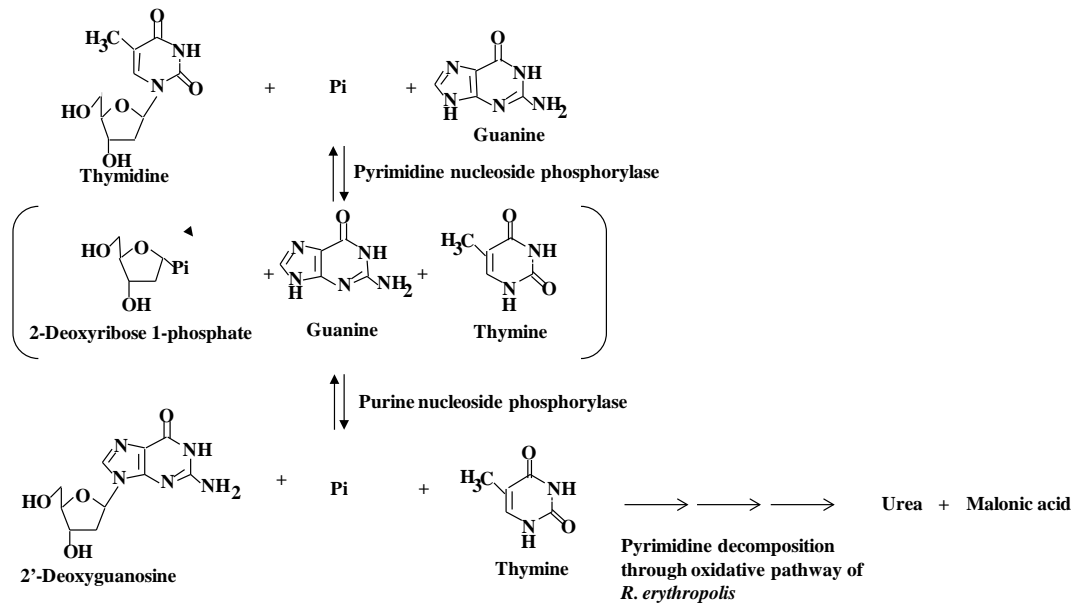


Fig. 2
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Figure legends

Fig. 1

Microbial metabolism of pyrimidine base.

Fig. 2

Equilibrium control of base exchange reaction for valuable nucleoside synthesis by oxidative pyrimidine degradation.